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DEVELOPMENTAL BIOLOGY OF THE EARLY CAMBRIAN CNIDARIAN *OLIVOOIDES*

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Abstract: Fossilized embryos afford direct insight into the pattern of development in extinct organisms, providing unique tests of hypotheses of developmental evolution based in comparative embryology. However, these fossils can only be effective in this role if their embryology and phylogenetic affinities are well constrained. We elucidate and interpret the development of *Olivooides* from embryonic and adult stages and use these data to discriminate among competing interpretations of their anatomy and affinity. The embryology of *Olivooides* is principally characterized by the development of an ornamented periderm that initially forms externally and is subsequently formed internally, released at the aperture, facilitating the direct development of the embryo into an adult theca. Internal anatomy is known only from embryonic stages, revealing two internal tissue layers, the innermost of which is developed into three transversally arranged walls that partly divide the lumen into an abapertural region, interpreted as the gut of a polyp, and an adapertural region that includes structures that resemble the peridermal teeth of coronate scyphozoans. The anatomy and pattern of development exhibited by *Olivooides* appears common to the other known genus of olivooid, *Quadrapyrgites*, which differs in its tetraradial, as opposed to pentaradial symmetry. We reject previous interpretations of the olivooids as cycloneuralians, principally on the grounds that they lack a through gut and introvert, in embryo and adult. Instead we consider the affinities of the olivooids among medusozoan cnidarians; our phylogenetic analysis supports their classification as total-group Coronata, within crown-Scyphozoa. *Olivooides* and *Quadrapyrgites* evidence a broader range of life history strategies and bodyplan symmetry than is otherwise commonly represented in extant Scyphozoa specifically, and Cnidaria more generally.

Key words: development, embryo, Cnidaria, Scyphozoa, Kuanchuanpu, Cambrian.

THOUGH there remains considerable debate over the timing of origin and diversification of animals (dos Reis *et al.* 2015), the tempo of early animal evolution is astonishing given the scale of innovation achieved (Erwin *et al.* 2011): the establishment of all phylum-level body plans that are sufficiently distinct that they are effectively defined by the limits of comparative anatomy (Bengtson 1986). Insights into the processes that brought about this remarkable episode in evolutionary history are afforded through comparative embryology of living animals, facilitating inference of the embryology of ancient ancestors and, indeed, into the evolution of development that brought about the origin of animal bodyplans. Inevitably, this approach is confused by the subsequent developmental evolution that serves to conflate homologies and convergences. Hence, the discovery of a fossil record of embryology from early in animal evolutionary history affords a more direct insight into the embryology of ancient ancestors, free from the confounding effects of half a billion years of subsequent evolutionary history.

Unfortunately, the evolutionary significance of the majority of fossilized embryos has yet to be realized because only disparate stages of embryology are preserved, providing insufficient evidence to constrain their phylogenetic affinity, or else insufficient evidence in order to make material comparisons to the embryology of living relatives (Donoghue *et al.* 2015). The olivooids, known from the early Cambrian Kuanchuanpu

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Fauna of South China, are a remarkable exception in that they are represented by a broad range of embryonic and post-embryonic stages of development preserved with great fidelity (Bengtson & Yue 1997; Yue & Bengtson 1999*a*). However, they have yet to achieve any material evolutionary significance principally because their phylogenetic affinities are so poorly constrained. This occurs for three principal reasons: (1) since Yue & Bengtson (1999*a*) a number of novel developmental stages have been described in disparate publications with little attempt to integrate these stages into a coherent model of development (Chen 2004; Hua *et al.* 2004; Steiner *et al.* 2004; Liu *et al.* 2007, 2008; Chen & Dong 2008; but see Li *et al.* 2007; Steiner *et al.* 2014); (2) the confidence with which component developmental stages have been attributed to *Olivooides* has been questioned (Steiner *et al.* 2004); and (3) the phylogenetic implications of each new report has invariably been considered independently of existing hypotheses and data. We attempt to remedy these shortcomings by providing a review of all putative developmental stages that have been attributed to *Olivooides*, describe a number of new developmental stages that contribute to the debate over the confidence with which existing stages have been allied, and consider the phylogenetic affinity and evolutionary significance of *Olivooides* in this light.

HISTORY OF RESEARCH INTO *OLIVOOIDES*

The name *Olivooides* was initially used by Qian (1977) for small, smooth spheroids of unknown nature among an assemblage of small shelly fossils from the Precambrian–Cambrian boundary of Shaanxi, China. Similar forms were subsequently recovered from other localities leading to the erection of new genera and species (Chen 1982; Yang *et al.* 1983; Luo *et al.* 1984; Xing *et al.* 1984). Authors compared these fossils to eggs in resting stages (Yue 1986) of animals (and more specifically to sponge gemmules; Yang *et al.* 1983) as well as to plants (Chen 1982).

Olivooides was first identified as a fossilized embryo by Bengtson & Yue (1997). Yue & Bengtson (1999*a*) subsequently described the development of *Olivooides* from probable cleavage embryos through embryonic development and were able to link the genus to co-occurring hatched conical fossils that had been previously known under the name *Punctatus* (= *Pyrgites*). While Yue & Bengtson (1999*a*) noted similarities to priapulids (in particular the pentaradial symmetry and overall appearance of the aperture), they argued for a cnidarian affinity because *Olivooides* has a single opening that presumably functioned as both mouth and anus, and because of similarities to the extinct conulariids which are thought to share synapomorphies with scyphozoan cnidarians (Werner 1966; van Iten, 1991, 1992*a*; van Iten & Cox 1992; Jerre 1994; van Iten *et al.* 2005, 2006).

Since the publication of Yue & Bengtson's (1999*a*) description of development in *Olivooides* a number of authors have described material that shows additional morphological details and developmental stages. Hua *et al.* (2004) described stellate embryos with a cap-like disc separated by a constriction from a larger ovoid body, interpreted as a blastodisc and yolk mass, respectively. This interpretation was effectively followed by Yao *et al.* (2011) who identified this as an instance of epibolic gastrulation, interpreting the stellate spines as ectodermal cells or else a cuticle secreted by migrating micromeres, and identifying the aperture as a blastopore.

Chen (2004) described a variety of new embryonic stages including a number of specimens with discs, which in some cases were folded into 10 lobes. Chen (2004) suggested that the pentaradial symmetry of *Olivooides* indicated that its phylogenetic affinities lay with the echinoderms. Steiner *et al.* (2004) identified *Pseudooides*, which occurs in the same samples as *Olivooides* and is also represented by embryonic stages. Since the majority of the associated cleavage stage embryos fall within the size range of *Pseudooides*, not *Olivooides*, Steiner *et al.* (2004) argued that they belong to *Pseudooides*. Li *et al.* (2007) figured a number of developmental stages of *Olivooides* including what they interpreted as two larval stages. Liu *et al.* (2006, 2007, 2008, 2009*b*) also illustrated well-preserved apertural lobes of the hatched animal, attempting to better resolve the developmental sequence from cleavage embryo through to thecate polyp adult. Li *et al.* (2007) corroborated the broad developmental sequence of embryo to theca described previously by Bengtson & Yue (1997; Yue & Bengtson 1999*a*, *b*), identifying *Quadrapyrgites* as a tetramerous equivalent of pentamerous *Olivooides*. Indeed, Liu *et al.* (2009*a*) went on to show commonalities in the developmental sequence of *Quadrapyrgites* and *Olivooides* extended into embryonic stages of development.

Chen & Dong (2008) used x-ray tomography to analyse the internal structure of *Olivooides*. They concluded that commonly preserved ovate internal structures are taphonomic, rather than original features of the biology of *Olivooides*. Chen & Dong (2008; following Yue & Bengtson 1999*a*, p. 184, figs 7–8) also argued that no new stellae are added during the post-embryonic stages of development. Hou *et al.* (2010) described embryos of *Olivooides* with an integument with conical, rather than stellate, surficial structures. Zheng *et al.* (2012*a*, *b*) also set out to more fully resolve the pattern of development of *Olivooides*, identifying a sequence of cleaving blastulas,

culminating in an embryo with a large blastocoel (though the crucial structures in these stages appear to be abiological diagenetic crusts). They interpret the aperture-bearing stages, with and without a stellate integument, as gastrula stages that ultimately transform into the 'Punctatus' theca stage through the development of striate tissue at the aperture that they interpret to have a shutter action capable of opening and closure.

Most recently insights have been provided into the internal anatomy of the embryonic developmental stages of *Olivooides* (Dong *et al.* 2013; Han *et al.* 2013; Yasui *et al.* 2013) and *Quadrapyrgites* (Han *et al.* in press). Tomographic analyses of the post-embryonic thecate stage by Yasui *et al.* (2013) revealed a vast open lumen occupied merely by a very short extension of the aperture, which they interpret as a gut. The limited extent of the gut and the apparent absence of tentacles led the authors to conclude that *Olivooides* had endosymbionts. Yasui *et al.* (2013) followed Yao *et al.* (2011) in identifying the stellate embryonic stage as a gastrula, and recorded the development of the mouth directly from the blastopore. Ultimately, they concluded that *Olivooides* is a stem-eumetazoan because it lacks many cnidarian characters and exhibits a unique bodyplan of thecal terminal addition built upon the chassis of its gastrula.

Dong *et al.* (2013) described *Olivooides* embryos with preserved internal anatomy, showing that the apertural lips extend internally, presumably representing preformed integumentary tissue prior to its out-folding to accommodate extension of the thecal tube of the post-embryonic stages. They also described a specimen that preserves extensive internal anatomy, which demonstrates that the external pentameral symmetry is a reflection of a more fundamental pattern of symmetry imposed on the internal anatomy. The preserved structures include a series of walls that were interpreted as the gastrodermis, preserving the course of radial canals, and including ridges that might represent supports for the mesenteries, though the abapertural end of the specimen is not preserved this region, opening to a central lumen within the preserved portion of the embryo. Dong *et al.* (2013) also attributed to *Olivooides* a co-occurring pentaradial structure interpreted as a strobilar fragment consisting of two adhering ephyrae, which would appear to settle phylogenetic debate on a cnidarian affinity for *Olivooides*. However, Steiner *et al.* (2014) questioned the association of this fossil with *Olivooides* and, indeed, whether it represents a medusoid stage. Steiner *et al.* went on to argue that the embryo with preserved internal structure is not compatible with a cnidarian affinity and plumbed instead for an affinity with cycloneuralians, drawing comparison to the loricae of loriciferans and of priapulid larvae, as well as the pentameral symmetry of the scalids of some scalidophorans, though *Olivooides* lacks scalids and a through gut. This interpretation was followed keenly by Liu *et al.* (2014*b*), but the preserved internal structures of *Olivooides* embryos are certainly no more compatible with a cycloneuralian affinity for which we should anticipate the presence of a vast undivided lumen inside the body wall.

Han *et al.* (2013) did not share the concerns of Steiner *et al.* (2014) in fitting a cnidarian bodyplan to the internal structures described by Dong *et al.* (2013). Indeed, they described specimens preserving precisely the same internal features, also attributed to *Olivooides*. The anatomy of *Olivooides* embryos and medusae of modern cubozoans are compared in great detail, leading ultimately to the hypothesis that *Olivooides* is a cubozoan. In particular, these specimens preserve the abapertural region (not seen in the material described by Dong *et al.* 2013), which appears to preserve the pharyngeal lumen and structural supports for the mesenteries.

Steiner *et al.* (2014) and Han *et al.* (in press) described the pattern of development of *Quadrapyrgites*, which they revealed to be like *Olivooides* in all respects, with the exception that it is tetramerous rather than pentamerous. Liu *et al.* (2014*a*) challenged the interpretation of both *Olivooides* and *Quadrapyrgites* as cycloneuralians, emphasizing the lack of a through gut in even the latest thecate stages, evident absence of bilateral symmetry, moulting, setae, scalids or spines, and differences in the topology of the growth zone. Liu *et al.* (2014*a*) also rejected the interpretation of *Olivooides* and *Quadrapyrgites* as cubozoan cnidarians (Han *et al.* 2013) on the basis that cubozoan polyps lack the mesenteries seen in *Olivooides*. Indeed, they highlighted the reduced polyp stage in living cubozoans, contrasting it with the apparent dominance of the interpreted polyp stage in *Olivooides* and *Quadrapyrgites*, which lasted for very many growth episodes. Ultimately, Liu *et al.* (2014*a*) drew affinity with hexangulaconulariids, conulariids and coronate scyphozoans. Han *et al.* (in press) perpetuated the cubozoan interpretation of olivooids in describing embryonic stages compatible with those attributed to *Quadrapyrgites* by Steiner *et al.* (2014). Importantly, they were the first to apply numerical cladistics to resolve the affinity of the olivooids among Cnidaria, concluding that the olivooids are stem-cubozoans. However, this is perhaps an inevitable consequence of interpreting the fossils following a cubozoan gestalt (cf. Donoghue & Purnell 2009).

Evidently, there are conflicting interpretations over the anatomy, development and affinity of the olivooids, *Olivooides* and *Quadrapyrgites*. Here we seek to review the development of *Olivooides*, the taxon whose anatomy is best known as a consequence of preserved internal features. We achieve this by drawing on existing knowledge supplemented with new material that provides a more finely resolved understanding of the morphogenesis of the anatomy preserved in the embryonic and post-embryonic

thecate stages. In this light we evaluate existing interpretations of the biology of the developmental stages, culminating in a consideration of the competing interpretative models and the phylogenetic affinity of *Olivooides* and, by implication, olivooids more generally.

MATERIAL AND METHOD

The fossil material analysed in this study is from the early Cambrian Kuanchuanpu Formation at the Shizhonggou section near Kuanchuanpu village, Ningqiang County, Shaanxi Province, China. Fossils were recovered by digestion of limestone in *c.* 10% acetic acid and separation from the insoluble residue by manual picking under a binocular microscope. Specimens were analysed using environmental scanning electron microscopy (SEM) and synchrotron radiation x-ray tomographic microscopy (SRXTM). SRXTM analyses were carried out at the X04SA Materials Science and X02DA TOMCAT beamlines of the Swiss Light Source, Paul Scherrer Institute, Villigen, Switzerland. We reanalysed micro-computed tomographic data (micro-CT) of two specimens, also from the Kuanchuanpu Formation, originally published in Han *et al.* (2013), and kindly provided by the authors. The figured material is reposited in the collections of the Geological Museum of Peking University, Beijing (GMPKU) and the Early Life Institute, Northwest University, Xi'an (ELISN). Three-dimensional computer modelling including the construction of external surfaces, segmentation of internal structures and 3D renderings of anatomical structures of specimens was conducted using the software Avizo v.8.0. Phylogenetic analyses were performed using paup 4.0a146 (Swofford 2002).

RESULTS

Cleavage and gastrulation stage embryos

Associated with the later developmental embryonic stages of *Olivooides* are specimens composed of two to several hundred compartments, interpreted as cleavage stage embryos (Bengtson & Yue 1997; Yue & Bengtson 1999*a*; Steiner *et al.* 2014; Fig. 1). Chen *et al.* (2004) attempted to reinterpret the compartments (Fig. 1E–F) as yolk pyramids, but this can be rejected in light of their geometry (Donoghue *et al.* 2006) and pattern of reductive division (Steiner *et al.* 2004). Many specimens exhibit a central space that, in most instances, is an artefact of incomplete preservation. However, in some specimens the surrounding cells are differentiated into inner and outer cell layers (Fig. 1F). These are gastrulae and the central space is the archenteron (Bengtson & Yue 1997; Yue & Bengtson 1999*a*; Donoghue *et al.* 2006). Descriptions of the earliest cleavage stages and of blastulae with a large blastocoel, are clearly based on poorly preserved remains whose critical structures are comprised of late diagenetic cement associated with void filling rather than with mineralization of original biological substrates. It is difficult to definitively link these specimens to *Olivooides* or other co-occurring taxa (Liu *et al.* 2007, fig. 9e; Zheng *et al.* 2012*a*, figs 1c–d, 2e–k, 3b–c, 2012*b*, figs 1b, d–g, 6b, d; Yasui *et al.* 2013, fig. 1b–c; Steiner *et al.* 2014, fig. 7.4).

Steiner *et al.* (2004) argued, on the basis of their size distribution, that the majority of cleavage embryos belong to the lifecycle of the enigmatic germ-band embryo *Pseudooides*, rather than that of *Olivooides*. While this is likely to be correct, specimens that fall within the size range of *Olivooides* (and outside that of *Pseudooides*) could well represent the cleavage stages of *Olivooides*. A number of the cleavage embryos show evidence of clustering of the component cells in a manner that suggests that the clusters reflect component cell lineages (Fig. 1B–D), while other cleavage embryos show no such pattern. This may reflect a heterogeneous assemblage of developmental stages from a number of different taxa (Yue & Bengtson 1999*a*; Steiner *et al.* 2014). However, the lack of specimens intermediate of cleavage and later embryonic stages that can readily be assigned to *Olivooides* renders it impossible, at present, to provide definitive insight into the cleavage and gastrulation stages of the development of *Olivooides*.

Aperture-bearing stellate embryos

The earliest developmental stages that can be assigned with confidence to *Olivooides* are spherical forms with a single pentaradial aperture (Figs 2A–C, 3A–C). We arbitrarily use the aperture as a datum for describing the anatomy of the fossils, defining an apertural–adapertural pole of pentaradial symmetry. The outer surface of the embryo may initially be effaced (Figs 2E–F, 3E–F), distinguished only by the rudiment of the aperture, however, it is possible that specimens that evidence such a stage are merely taphonomic variants (e.g. endocasts) of embryos that are regularly encased not only by a fertilization envelope but also by a continuous sheet of tissue that is developed into stellae (spines with radiating folds that impart a star-shaped appearance) that are 40 μm in length on the lower surface to 70 μm or more on the upper surface (Figs 2A–D, G–I, 3A–D, G–I, 4A–B).

Early embryonic development is most readily characterized by changes to the aperture (Fig. 2). In the earliest post-cleavage stage that we recognise, the aperture is comprised of five lobes and composed of stellate tissue (Figs 2A, 3A). Additional intercalary lobes develop subse-

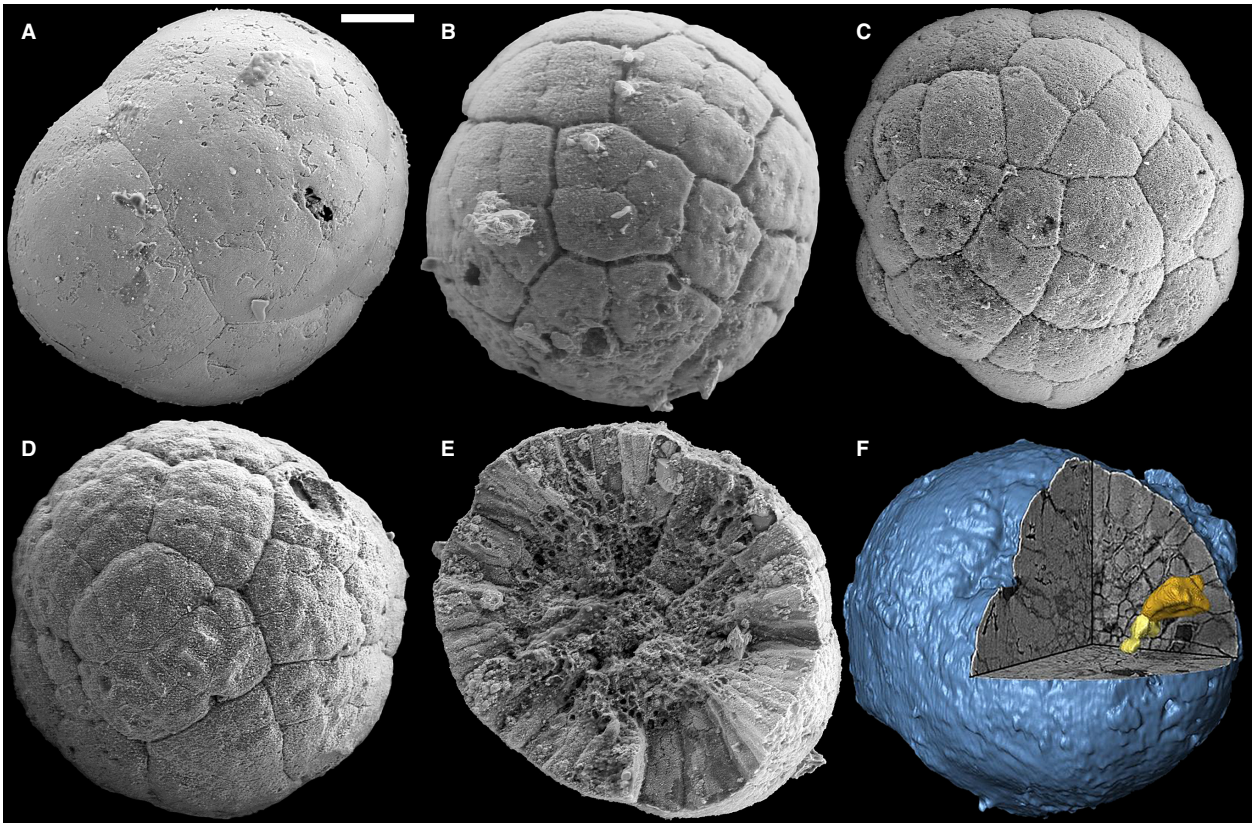

FIG. 1. Cleavage stages associated with developmental stages of *Olivooides* and *Pseudooides*. A, four-cell embryo (GMPKU3118). B, embryo in which blastomeres appear to be grouped according to their lineage of division, emphasized by differences in the preservation or chemistry of the mineral replacing the cell walls (GMPKU3119). C, comparable stage of cleavage to B, but showing different preservation (GMPKU3080, reproduced from Dong *et al.* 2013, fig. 1a). D, a later stage of cleavage showing the same phenomenon of cell clustering as in B (GMPKU3120). E, blastula or gastrula, fractured during recovery, showing the long, tapering cells that comprise the outer part of the embryo (GMPKU3121). F, rendering of a tomographic scan of an embryo that appear to have undergone gastrulation since there are cells with a differentiated geometry and volume comprising the outer and inner layers (reproduced from Donoghue *et al.* 2006, fig. 1d). Relative scale bar represents: A, 53 μm; B, 63 μm; C, 65 μm; D, 48 μm; E, 75 μm; F, 105 μm. Colour online.

quently, obeying the original fivefold symmetry (Figs 2B, 3B). A wrinkled tissue begins to emerge from within the lobes of the aperture and extends down the outer surface, grading imperceptibly with the stellate ornament that otherwise continues to extend over the surface of the embryo (Figs 2C, 3C). Through these stages of development, the apertural region is raised relative to the residual surface of the embryo (Figs 2A–C, 3A–C).

In subsequent development, the apertural region expands from about one-third to four-fifths of the diameter of the embryo in apertural view (Fig. 2D–I). The five principal lobes of the aperture are broader than at earlier developmental stages and are seen eventually to overlie one or two subordinate intercalary rays (Figs 2D–F, 3D–F). However, it is clear from this pattern that the lobes are merely folds in the outer wall that comprises the aperture, accommodating sufficient tissue to eventually unfold to the diameter of the embryo. At around this stage, a constriction develops, gradually distinguishing the main body of the embryo from an apertural disk that is composed mainly of unornamented or wrinkled tissue (Figs 2F–G, 3F–G). The aperture opens, the rays unfolding (Fig. 2F, I), to release the striate tissue that characterizes post-embryonic stages (Figs 2H–I, 3H–I, 4F–K), before the aperture again closes (Fig. 4I, K). Such specimens provide definitive evidence of the developmental link between the embryonic stages traditionally assigned to *Olivooides* (senior synonym) and *Punctatus* and *Pyrgites* (junior synonyms of *Olivooides*; Yue & Bengtson 1999*a*).

Through these developmental stages, the stellate tissue is preserved in different states of tension and it is unclear whether the appearance of folds and annulations (Figs 3D–G, 4A–B, F–K) reflect the increasing surface area of unfolding integument, or the taphonomic collapse of internal supporting structures. Nevertheless, when the stellate tissue is under less tension it appears to show

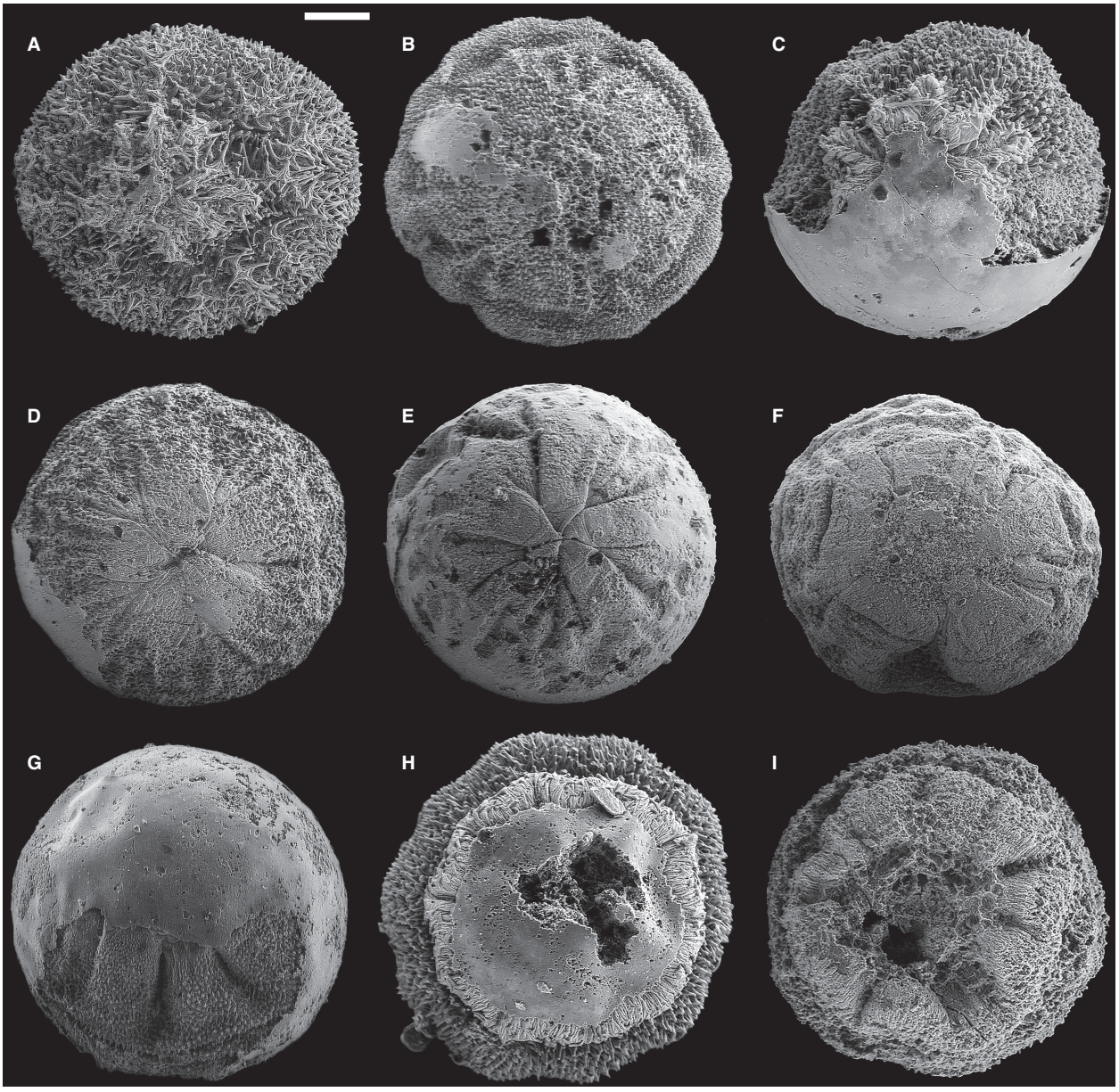

FIG. 2. Embryonic stages of *Olivooides* development in apertural view. A, embryo with stellate periderm in which the aperture has only begun to develop (GMPKU3122). B, embryo in which the principal and accessory rays of the aperture have developed (GMPKU3083, reproduced from Dong *et al.* 2013, fig. 1f). C, embryo showing the aperture with wrinkled tissue (GMPKU2315). D–I, embryos showing successive stages of aperture opening, the striate tissue has begun to develop in the folds around the aperture in specimens H and I (D, GMPKU3123; E, GMPKU3124; F, GMPKU3125; G, GMPKU3126; H, GMPKU3127; I, GMPKU3084). Relative scale bar represents: A, 122 μm; B, 126 μm; C, 112 μm; D, 142 μm; E, 148 μm; F, 149 μm; G, 152 μm; H, 110 μm; I, 124 μm.

external impressions of features of internal anatomy. For instance, an early developmental stage reveals that a cap-shaped structure and the five broad principal rays are evident internally (Figs 2B, 3B) before they are manifest externally (Figs 2D–I, 3D–I). Many specimens preserve ambital furrows in the stellate tissue perpendicular to the radial pole (Fig. 3D–G). In some instances, the ambital furrows are deflected outwards by, alternately, broad and narrow internal structures aligned parallel to the radial pole (Fig. 3F–G), and confluent with the five principal broad rays and the ridge between the paired subordinate intercalary rays at the aperture (Fig. 3F–G). Yue & Bengtson (1999*a*) suggested that these furrows were rudiments of the ambital rings that are apparent in post-embryonic stages (Figs 4I–K, 5A–B). However, they appear to be transient structures or may merely reflect decay of

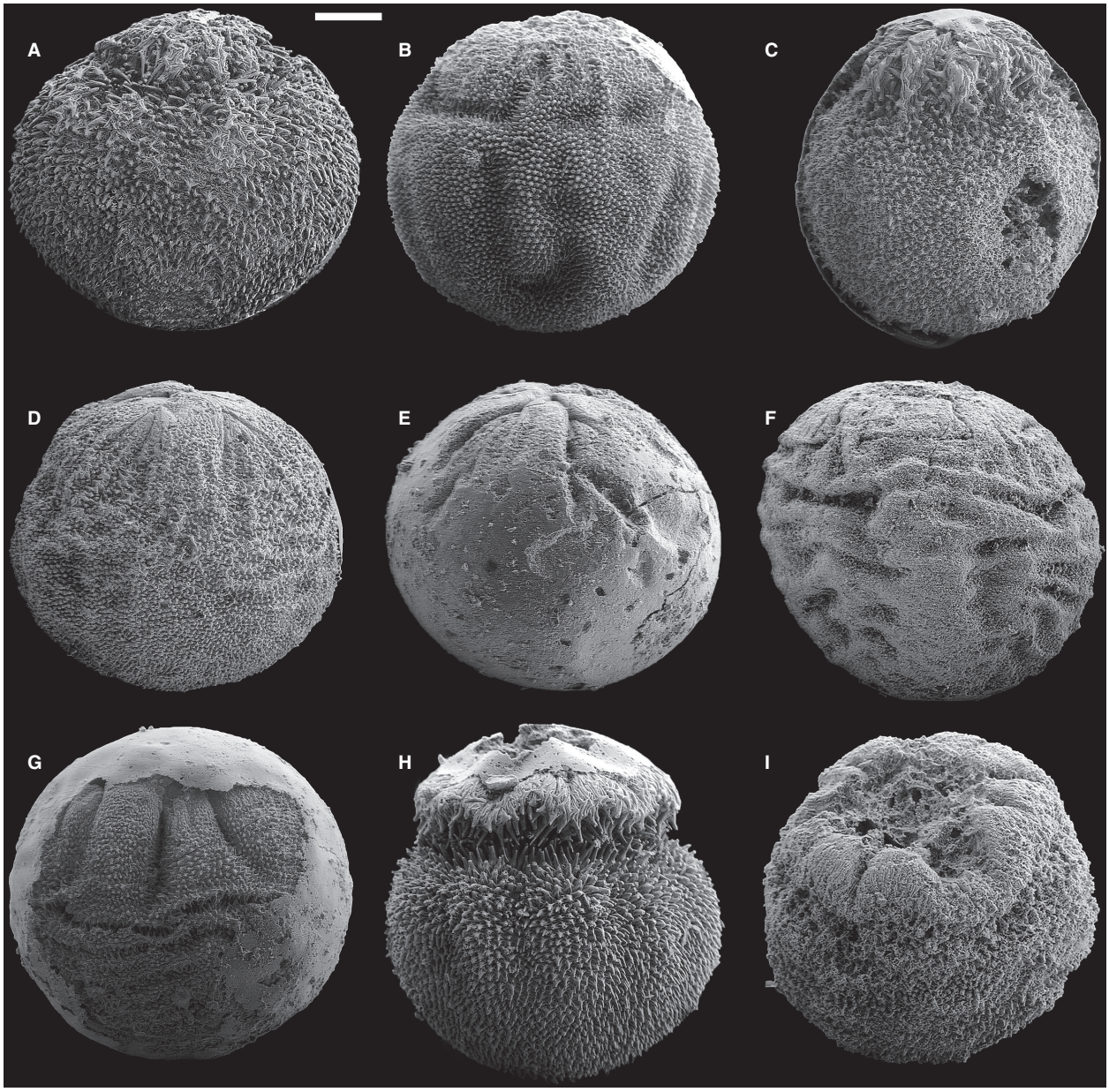

FIG. 3. Embryonic stages of *Olivooides* development in lateral aspect. Same specimens as in Figure 2, but presented in lateral aspect. A, embryo with stellate periderm in which the aperture has only begun to develop (GMPKU3122). B, embryo in which the principal and accessory rays of the aperture have developed (GMPKU3083, reproduced from Dong *et al.* 2013, fig. 1e). C, embryo showing the aperture with wrinkled tissue (GMPKU2315). D–I, embryos showing successive stages of aperture opening, the striate tissue has begun to develop in the folds around the aperture in specimens H and I; D, GMPKU3123; E, GMPKU3124; F, GMPKU3125; G, GMPKU3126; H, GMPKU3127; I, GMPKU3084. B, I, reproduced from Dong *et al.* 2013, fig. 1d–e. Relative scale bar represents: A, 124 μm; B, 132 μm; C, 129 μm; D, 148 μm; E, 148 μm; F, 149 μm; G, 158 μm; H, 118 μm; I, 127 μm.

internal anatomy since they are too numerous (Fig. 3F) and are often not present in later embryonic stages (Fig. 3H–I).

The aperture-bearing embryonic stage has been interpreted, in whole or in part, as representing gastrulae by a number of authors (Hua *et al.* 2004; Liu *et al.* 2007; Yao *et al.* 2011; Li *et al.* 2012; Yasui *et al.* 2013; Steiner *et al.* 2014). In particular, Hua *et al.* (2004) interpreted the stage with the apertural disc-like structure (Figs 2H–I, 3H–I) as a blastodisc and the remainder of the embryo as the yolk body. Liu *et al.* (2007) interpreted the aperture as a blastopore, as did Yao *et al.* (2011) who also viewed the disc as a yolk plug, rationalizing the surface stellae as ectodermal cells, or else the folding of cuticle secreted by

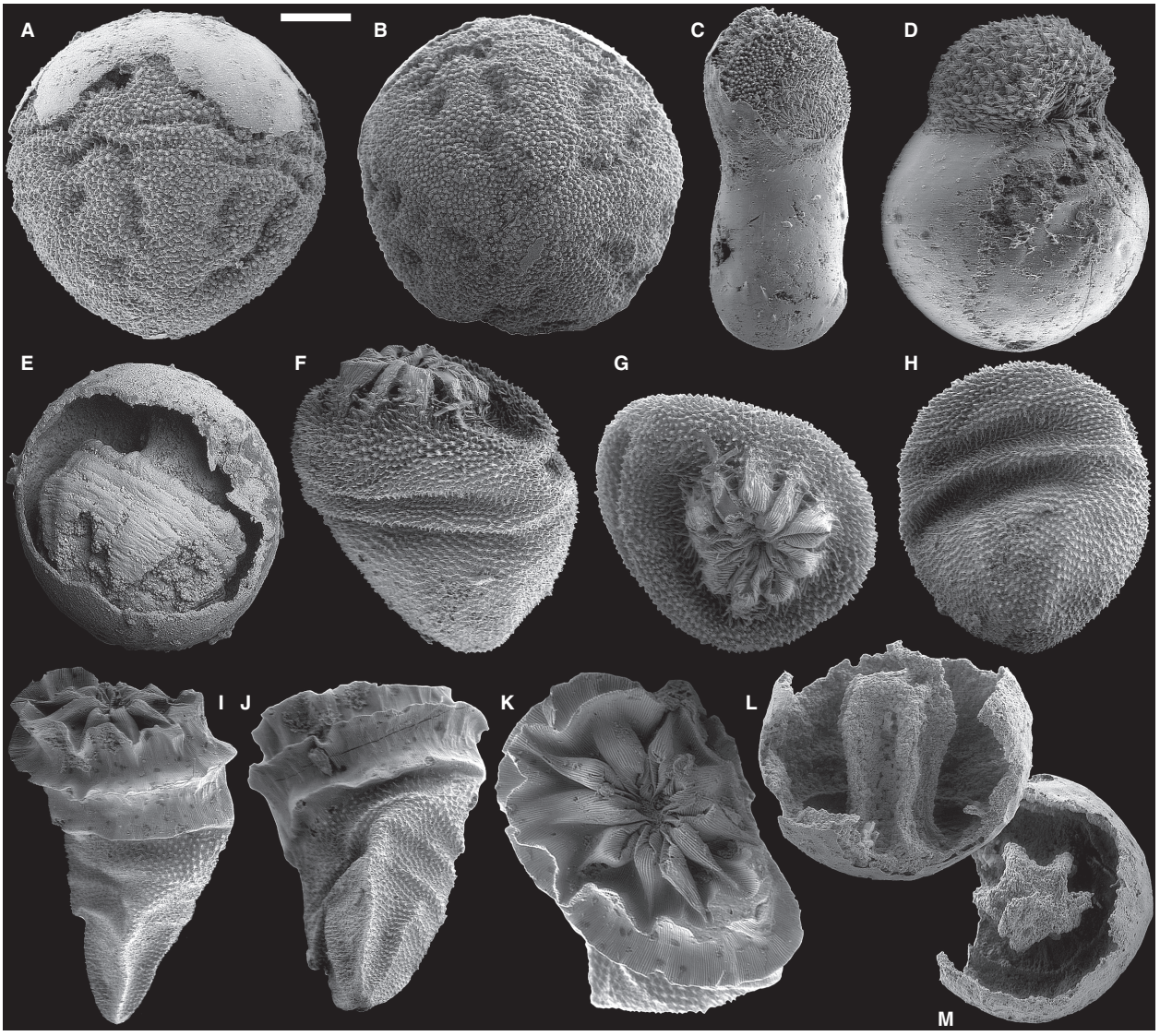

FIG. 4. Late embryonic and hatching stages of *Olivooides* development. A–B, embryo displaying radial and ambital furrows, reflecting internal anatomy (GMPKU3128). C, elongate hatching embryo (GMPKU3130). D, hatching embryo in which the embryo protrudes from the fertilization envelope (GMPKU3129). E, thecate morphology preserved within its fertilization envelope; only remnants of the stellate tissue remain (GMPKU3085, reproduced from Dong *et al.* 2013, fig. 1g). F–H, hatchling displaying on weak annulation and pentagonal base (GMPKU2316). F, lateral aspect. G, apertural view. H, oblique view of the pentagonal base of the developing theca. I–K, young theca showing its annulation and aperture comprised of striate tissue. I, lateral aspect. J, oblique view of base. K, aperture (GMPKU2317, reproduced from Dong *et al.* 2013, fig. 1h, i, j). L–M, embryo preserving possible supports for mesenteries. L, in lateral aspect. M, in apertural view (GMPKU3088, reproduced from Dong *et al.* 2013, fig. 2g–h). Relative scale bar represents: A, 151 μm; B, 149 μm; C, 204 μm; D, 183 μm; E, 217 μm; F, 208 μm; G, 212 μm; H, 225 μm; I, 388 μm; J, 348 μm; K, 257 μm; L, 192 μm; M, 211 μm.

migrating micromeres. This interpretation of the aperture as the blastopore and future mouth was followed by Yasui *et al.* (2013). Steiner *et al.* (2014) implicitly followed suit, attributing specimens with the pentaradial aperture as representing embryonic stages as early as a late blastula, and those in which stellae are manifest, as gastrulae.

We reject these interpretations since the blastodisc, as an embryonic structure, is present usually from the first cleavage stages and none of the cleavage embryos associated with *Olivooides* (nor, indeed, any of the cleavage embryos recovered from the deposit) exhibit a blastodisc. Furthermore, the evidential growth and reorganization of the wrinkled and stellate tissue (Figs 2G–I, 3G–I) is incompatible with it being a yolk body. The disc-like structure is composed of stellate or wrinkled or striate tissue (Figs 2H–I, 3H–I), rather than cleaving cells as would be expected under the blastodisc model. Indeed, these tissues are a consequence of gastrulation having

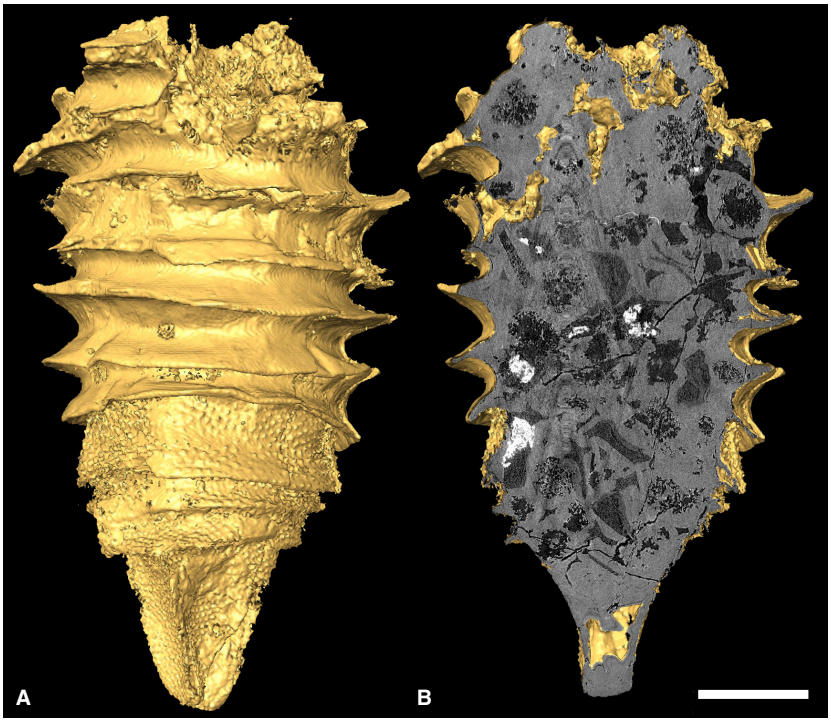

FIG. 5. Computed tomographic models of the post-embryonic thecate stage of *Olivooides* derived from SRXTM characterization of the fossil (GMPKU2311). A, surface model showing the stellate and striate tissue comprising the abapertural and adapertural portions of the theca, respectively. B, intersecting orthoslice showing the internal structure including void-filling cements and sphaeroidal structures. Scale bar represents 500 μm. Colour online.

occurred long before, not evidence of gastrulation occurring at the embryonic stage preserved. If this were not enough, there is clear evidence that organogenesis has occurred much earlier, manifested both in the expressions of internal anatomy as well as ambital and radial bulges in the stellate tissue, and direct evidence of anatomy preserved inside the embryos (Dong *et al.* 2013; Han *et al.* 2013).

The internal structures preserved in the pentaradial aperture-bearing embryos described by Dong *et al.* (2013) and Han *et al.* (2013) are compatible. Their size range indicates that they are embryos of *Olivooides multisulcatus* (Steiner *et al.* 2014), excluding the possibility that they could be different species. Previously, these specimens have been interpreted almost entirely from tomographic slices (Dong *et al.* 2013; Han *et al.* 2013), in which it can be difficult to infer three-dimensional morphology, relationships among anatomical structures and, indeed, to discriminate preserved biological structure from artefacts of later diagenetic mineralization (Dong *et al.* 2013; Han *et al.* in press). We sought to further clarify the preserved anatomy by segmenting it using computed tomography, free of structures that we interpret as mineralized remains of decayed structures, including remains of the 'polygonal axial structure' described by Dong *et al.* (2013), and later diagenetic mineralization. Biological structure is preserved in a mineral phase that exhibits relatively high x-ray attenuation, and the features exhibit distinct margins. Our tomographic models are presented in Figures 6–8.

Inside the integument, there is an inner and an outer wall (Figs 6C, 7D, 8D) defining an internal lumen that is divided by five internal ridges aligned approximately parallel to the apertural–abapertural (radial) axis. These ridges are equidistant from each other and link the two walls (Fig. 6D–F, white arrows). Additionally, in between the inner and outer walls, there are short cross walls some of that are elongated extensions from the ridges (Figs 6F, 7G, white arrowheads), while some are spurs of the outer wall that extend into the lumen (Figs 6C, 7D, grey arrows). At the apertural margin, the outer wall folds and modifies into five principal apertural lobes, each with a pair of smaller intercalary lobes (Figs 6A, 7A), mirroring the structure of the outer stellate and striate tissue seen in other specimens (Figs 2–4). The apertural lobes can extend abaperturally from the inner wall, forming five pairs of apertural ridges in at least one specimen (Fig. 6C, grey arrowhead). The tips of the lobes protrude towards the main lumen, delimited by the inner wall. The outer wall exhibits longitudinal protuberances that are aligned with the intercalary lobes and extend towards the abapertural pole (Figs 6A–B, 7C, 8B–C).

The aperture extends from 40 to 60% of the radial diameter and it opens into the main lumen within the inner wall, comprising the main volume of the embryo. The lumen is partially divided into three distinctive regions by walls that project transversally to the inner wall (Figs 6C, 7D, 8D, black arrows). Arranged at approximately one-quarter of the length of the apertural–abapertural axis, the first of these regions is comprised of five

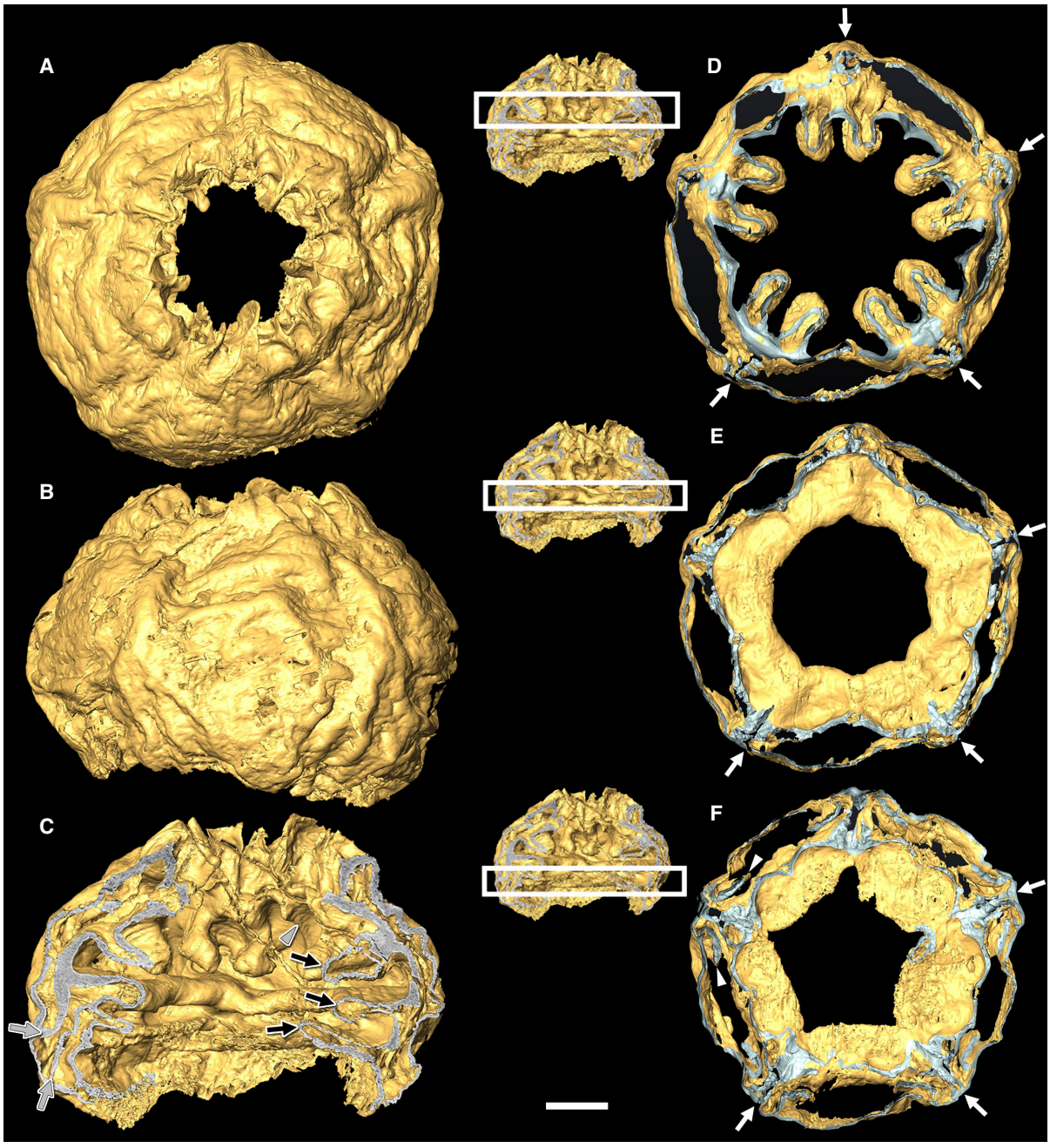

FIG. 6. Computed tomographic models of preserved internal anatomy in an embryo of *Olivooides* derived from SRXTM characterization of the fossil which does not preserve stellate tissue and abapertural part (GMPKU3089). A, apertural view. B, lateral aspect. C, lateral aspect with virtual slice through the embryo revealing: the inner and outer walls (which would have been located inside the stellate periderm); the development of the inner wall into the peridermal teeth (top black arrow) and two infoldings of the inner wall that extend into the lumen (bottom two black arrows); an internal ridge extending from the aperture (grey arrowhead); the presence of cross walls that spur from the outer wall, eventually linking the inner and outer wall (grey arrows). D–F, transversal sections of the peridermal teeth and two other infoldings of the inner wall as seen in C; connecting the inner wall to the outer wall there are five ridges forming a pentagonal shape (white arrows). D, transversal section of five pairs of peridermal teeth protruding towards the lumen. E, transversal section of the second abradial structure: a continuous wall exhibiting 10 small protuberances. F, transversal section of the third wall that protrudes from the inner wall into the lumen, defining a central pentagonal aperture; in between the inner and outer wall it is possible to observe cross walls originating from the five internal ridges (white arrowheads). Three-dimensional models were constructed from original tomographic data published in Dong *et al.* (2013). Relative scale bar represents: A, 93 μm; B, 94 μm; C, 92 μm; D, 116 μm; E–F, 111 μm. Colour online.

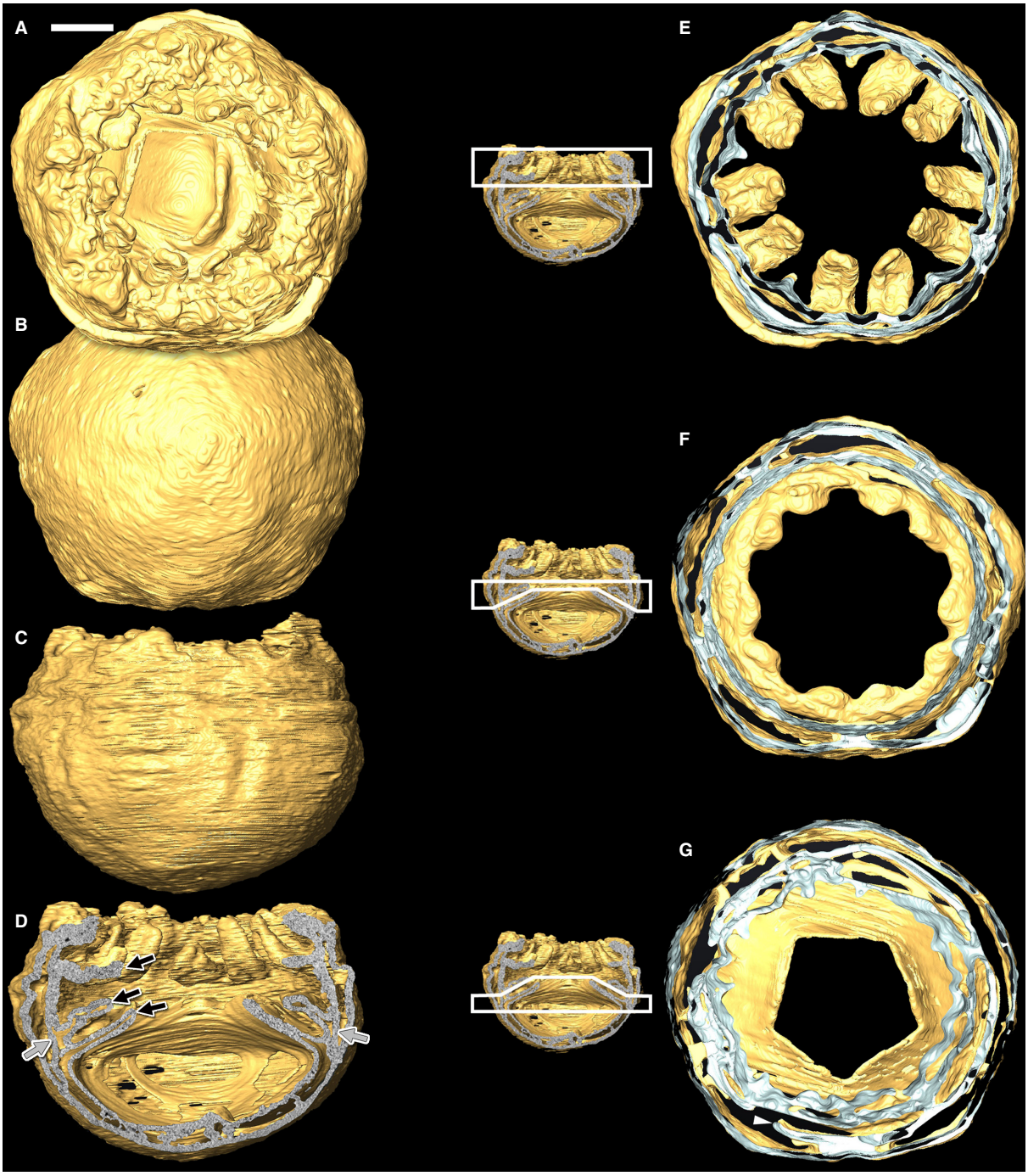

FIG. 7. Computed tomographic models of preserved internal anatomy in an embryo of *Olivooides* derived from micro-CT characterization of the fossil (ELISN108-343). A, apertural view. B, abapertural view. C, lateral aspect. D, lateral aspect with tomographic cutaway revealing the inner and outer walls within the stellate periderm (not preserved) and the development of the inner wall into the peridermal teeth (top black arrow), a second continuous wall and the third wall protruding into the lumen (bottom two black arrows) and forming and undivided abapertural space; grey arrows indicate spurs extending between the cross walls. E–G, transversal sections of the peridermal teeth and two other infoldings of the inner wall as seen in D. E, transversal section of five pairs of peridermal teeth protruding towards the lumen. F, transversal section of the continuous wall, exhibiting 10 small protuberances. G, transversal section of the third wall that protrudes from the inner wall into the lumen, defining a central pentagonal aperture; in between the inner and outer walls it is possible to observe cross walls originating from the five internal ridges (white arrowhead). Three-dimensional models were constructed from original tomographic data published in Han *et al.* (2013). Relative scale bar represents: A–D, 92 μm; E, 83 μm; F, 92 μm; G, 90 μm. Colour online.

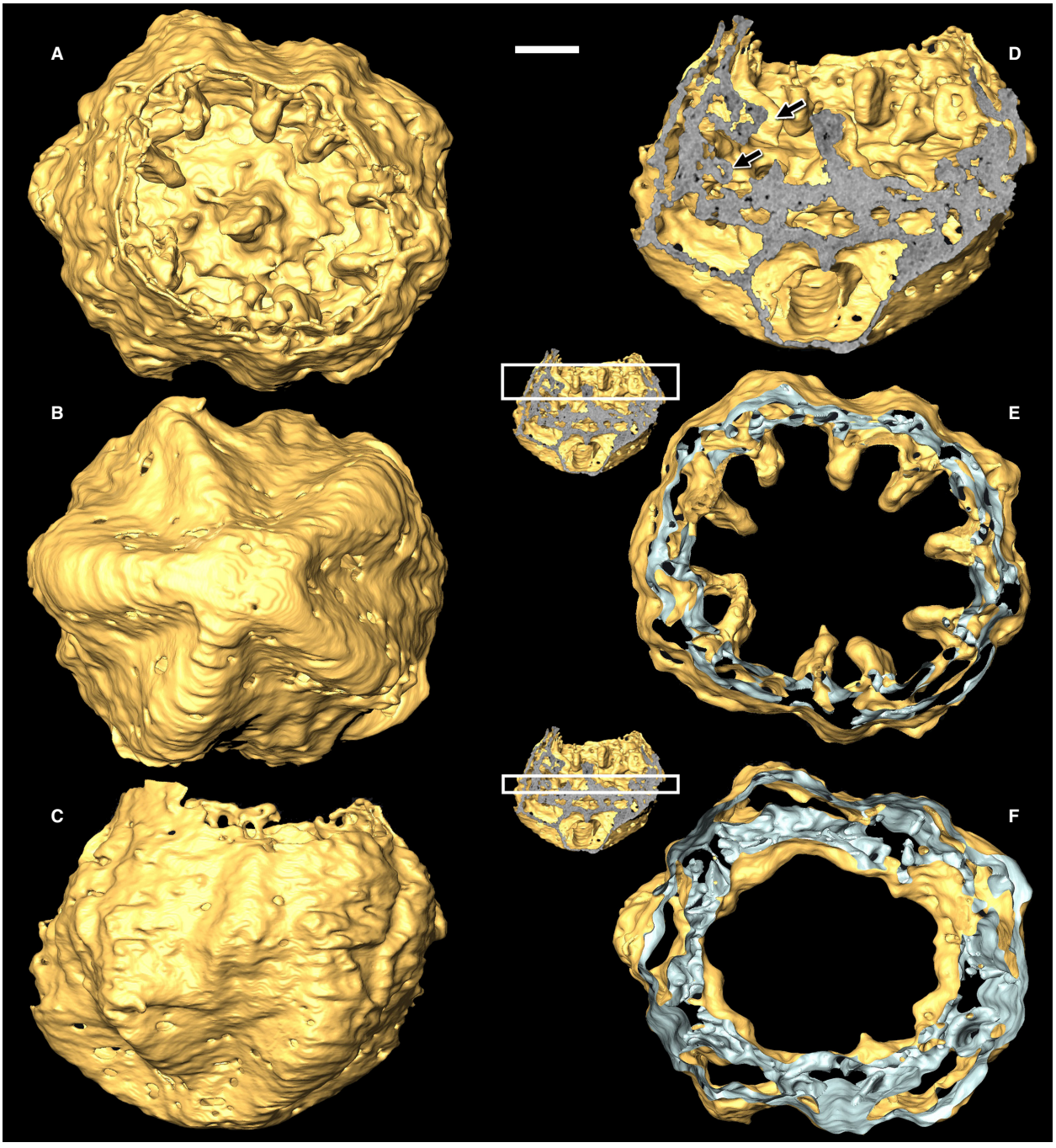

FIG. 8. Computed tomographic models of preserved internal anatomy in an embryo of *Olivooides* derived from micro-CT characterization of the fossil (ELISN31-5). A, apertural view but the main and intercalary apertural lobes are not preserved. B, abapertural view. C, lateral aspect. D, lateral aspect with tomographic cut-away revealing the inner and outer walls within the stellate periderm (not preserved) but anatomy less distinct from space filling diagenetic mineralization; black arrows indicate the five pairs of peridermal teeth (top) and the continuous wall extending from the inner wall into the lumen (bottom). E–F, transversal sections of the two visible walls extending from the inner wall. E, transversal section of five pairs of peridermal teeth protruding towards the lumen. F, transversal section of the continuous wall, exhibiting 10 small protuberances. Three-dimensional models were constructed from original tomographic data published in Han *et al.* (2013). Relative scale bar represents: A–B, 91 μm; C, 93 μm; D, 97 μm; E, 88 μm; F, 90 μm. Colour online.

pairs of pentaradially arranged abradial lobes (Figs 6C–D, 7D–E, 8D–E). These exhibit some slight variation in morphology, extending in breadth from the inner wall in some specimens (Figs 7D, 8D), extending from the internal apertural ridges in others (Fig. 6C); we interpret these as reflecting slightly different developmental stages since their position depends on the extending aperture. The second structure projects from the inner wall at approximately midway along the apertural–abapertural axis forming a continuous wall, exhibiting 10 minor (but no less distinct) protuberances that are aligned with the pentaradially arranged pairs of lobes (Figs 6C, E; 7D, F; 8D, F). The third structure is another continuous wall that extends to approximately two-thirds of the diameter of the central lumen, almost enclosing the abapertural lumen by a central pentagonal aperture (~140 μm to a side; Figs 6C, F; 7D, G) which is the remaining undivided abapertural space (Fig. 7D). An amorphous mineralized mass sometimes occupies the main lumen (e.g. Fig. 8A, D), where it was not possible to distinguish from the biological material; it is possible that this represents the decayed remains of the polygonal radial structure described by Dong *et al.* (2013; Fig. 4L, M), but as it is structureless and anatomically uninformative, we did not include it in our segmented model (Figs 6–7).

If nothing else, these preserved remains of internal anatomy demonstrate that the stages exhibiting the pentaradial aperture, which is related intimately with the development of the external integument, are very late embryologically, well beyond gastrulation, within the organogenesis phase of embryological development. Whether or not the pentaradial aperture develops from the blastopore, as has been argued explicitly or implicitly (Yasui *et al.* 2013) is a moot issue that cannot be determined on the basis of the available evidence; the key point is that it is not the blastopore.

Hatching

Embryonic stages demonstrate that the pentameral organization, characteristic of post-embryonic stages, was established in the embryo. A small number of specimens represent the hatching stage itself. In one specimen the embryo is preserved stretching the fertilization envelope (Fig. 4C), while in others the embryo protrudes through a rent in the envelope (Fig. 4D); the protruding portion of the embryo is composed of stellate tissue and the aperture is positioned at the margin of the rent in the fertilization envelope (Fig. 4D). The plastic behaviour of the stellate integument (and presumably the internal organs) demonstrates that no part of the embryo was mineralized or sclerotized in life. However, the stage of morphogenesis at hatching is seen to vary since, in at least one specimen, the aperture has opened fully to adopt the morphology of the initial 'Punctatus' stage that has been envisaged as post-embryonic, though it remains in its fertilization envelope (Fig. 4E).

Post-embryonic development

This stage is represented by the conical forms assigned previously to the genera 'Punctatus' and 'Pyrgites'. Yue & Bengtson (1999*a*) argued that the 'Punctatus' stage belonged to the lifecycle of the same animal as the embryonic *Olivooides* forms by demonstrating that both have striate tissue surrounding the aperture and stellate tissue on the remainder of the specimen. The discovery of a 'Punctatus' stage preserved *in ovo* (Fig. 4E) appears to provide further evidence of the link between classical 'Olivooides' and 'Punctatus' stages of development. This specimen does not preserve the stellate tissue characteristic of both stages, however, though it exhibits the characteristic pentaradial and interradial bulges in the wall seen in specimens that preserve the stellate tissue (Figs 3B, F, 4A, B, 5A) and in specimens that preserve internal anatomy (Figs 6–8).

Specimens representative of post-embryonic development (Figs 4F–K, 5) that exhibit features found in both the embryonic stages and the later conical stages, provide definitive evidence of a link between the two. The specimen in Figure 4F–H is only slightly larger than the hatching embryos shown in Figure 4C–D. Stellae cover the entire specimen apart from the folded apertural region where the aperture is raised into 10 lobes that are composed of striate tissue and surround a central orifice. The specimen is only weakly conical in comparison to larger specimens and the 10 pentaradially arranged ridges observed around the apices of more advanced post-embryonic stages, are absent. It is not strongly annulated although two nascent annulations are present. With the description of this specimen few differences remain between the final embryonic stages and the first conical stages of the *Olivooides* life cycle. This not only increases the security with which the two developmental stages can be linked, but also strengthens the evidence against an intervening free larval phase, to the extent that it can be excluded as a possibility.

The later parts of the conical developmental stage (Figs 4I–K, 5A) have been described well elsewhere (Conway Morris & Chen 1992; Steiner *et al.* 2014) and so only a brief description is given here. The cone has strong, regular annulations (Figs 4I–J, 5A). The five ridges comprising the conical abapertural region, which is increasingly well developed in larger specimens, radiate from the apex of the cone and reach as far as the first (as counted from the apex) annulation (Figs 4I–J, 5A; see also fig. 2a–b of

Yue & Bengtson 1999*a*); in this portion the cone diverges at approximately 60–90°. After the first annulation the test diverges less steeply at around 20° (Figs 4I–J, 5A). The apical portion of the cone as far as the fourth or fifth annulation is ornamented with stellae approximately 50 μm in length and identical in form to those found in the embryonic stages of development. The remainder of the cone is ornamented by longitudinal striae identical in appearance to those observed in the latest pre-hatching embryos. The proportion of striate tissue increases with the size of the specimen. While small specimens have striae only in the folds around the aperture, in large specimens striae can cover as much as two-thirds of the specimen (Fig. 5A). As Yue & Bengtson (1999*a*) concluded, this indicates that growth took place by the addition of the striate integumentary tissue, as we have described for the emergence and unfolding of the stellate, wrinkled and striate integument at the aperture of the embryo. Thecate stages occur with either an entirely open or closed aperture, though Steiner *et al.* (2014) argued that open-ended thecae are broken and the closed aperture reflects the natural condition. Given the overwhelming evidence for the formation of the integument within the embryo and theca, and for its release at the aperture, it is evident that the aperture was capable of opening to the diameter of the embryo and theca and, indeed, this is demonstrably the case in the embryonic stages. Thus, while many thecae with open apertures may well be broken, it does not follow that all open thecae are broken.

Nothing is known of the internal anatomy of the theca stage (Fig. 5A–B). Chen & Dong (2008) showed that the theca is frequently filled with sphaeroidal structures (Fig. 5B) that are of diagenetic origin and do not reflect the original biology of the organism, although the mineral fabric appears to have grown from a regular organic substrate (Dong *et al.* 2013). Yasui *et al.* (2013) identified a small blind gut attached to the base of the aperture, leaving the theca otherwise wholly unoccupied. However, this interpretation is clearly incompatible with the internal anatomy described from the embryonic stages (Dong *et al.* 2013; Han *et al.* 2013) and it appears, rather, as part of the postmortem diagenetic mineral lining of the theca, which clearly extends to the three adapertural annular rings.

Free-living stage

Dong *et al.* (2013) described a unique star-shaped specimen (Fig. 9) and interpreted it as a pair of closely adpressed five-arm ephyra larvae, with arms of adjacent individuals intercollating one another (Fig. 9A–B). On the basis of its unusual pentaradial symmetry, they interpreted this specimen as evidence of juvenile medusae, produced through strobilation, constituting part of the life cycle of *Olivooides*. While Han *et al.* (2013) and Yasui *et al.* (2013) accepted these interpretations, Steiner *et al.* (2014) queried whether the fossils belong to *Olivooides,* since they are smaller, other pentaradial fossils co-occur in the strata and symmetry alone, they argued, does not substantiate a link with *Olivooides*.

DISCUSSION

Olivooides has been the subject of a lively debate over its affinity since its discovery, but we will limit our discussion to those clades that have been considered since it

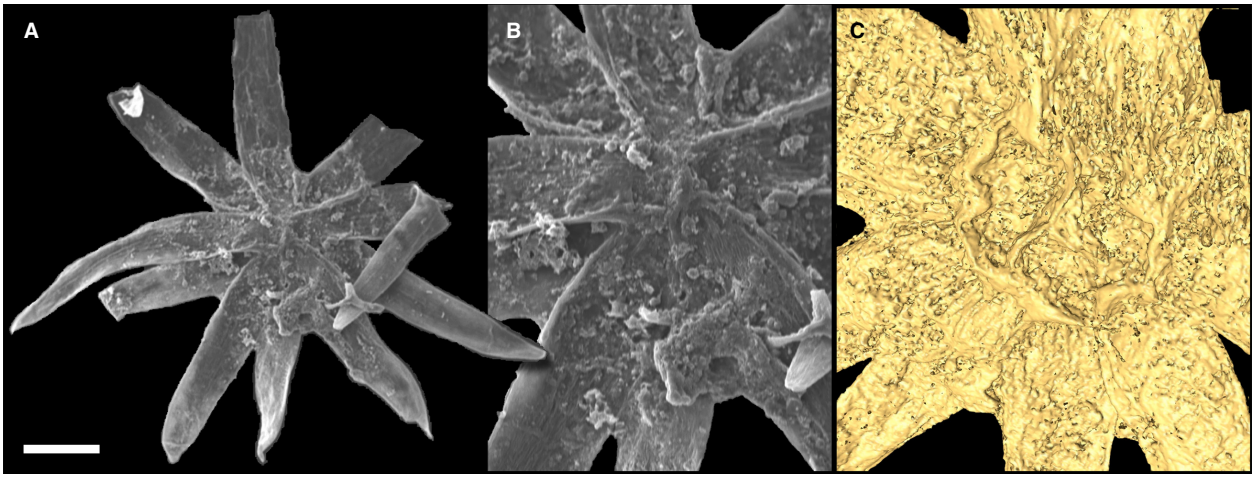

FIG. 9. A–C, possible five-arm ephyra larva associated with *Olivooides* (GMPKU 3090, reproduced from Dong *et al.* 2013, fig. 4a–c). A, abapertural view, with arms of adjacent individuals intercollating one another. B, detail of the abapertural surface. C, detail of the apertural surface based on computed tomography. Relative scale bar represents: A, 68 μm; B, 37 μm; C, 39 μm. Colour online.

was recognized as an embryo and associated with the 'Punctatus' thecate post-embryonic stage. Most authors have considered *Olivooides* to be a cnidarian, principally because the thecate stage had previously been allied to the conulariids (Conway Morris & Chen 1992), which have generally been considered cnidarians (Leme *et al.* 2008). However, *Olivooides* has also been considered a stem-eumetazoan (Yasui *et al.* 2013), a cycloneuralian (Steiner *et al.* 2010, 2014) and an echinoderm (Chen 2004). We will not consider the echinoderm hypothesis further, as it is based on nothing more than pentameral symmetry; it has been roundly rejected (Dong *et al.* 2013; Han *et al.* 2013; Yasui *et al.* 2013; Liu *et al.* 2014*a*) and can no longer be considered a live hypothesis. Steiner *et al.* (2014) also considered and rejected affinities to multifarious fossil problematica that co-occur in samples with olivooids, along with living Cycliophora; we see no reason to revisit any of these hypotheses and will instead limit our discussion to consideration of affinity to Cycloneuralia (Steiner *et al.* 2006, 2010, 2014), stem-Eumetazoa (Yasui *et al.* 2013) and Cnidaria (Bengtson & Yue 1997; Yue & Bengtson 1999*a*, *b*; Chen & Dong 2008; Dong *et al.* 2013; Han *et al.* 2013; Liu *et al.* 2014*a*).

Cycloneuralian affinity

Bengtson & Yue (1997) and Steiner *et al.* (2010, 2014) have drawn comparisons between *Olivooides* and the cycloneuralians, principally based on the similarities of the loricae of lorificerans and larval priapulids to the thecate post-embryonic stage of *Olivooides* development. Bengtson & Yue (1997) quickly dismissed this as a superficial similarity since there is no evidence of an anus in the theca of *Olivooides*. Steiner *et al.* (2014) highlighted the fact that preloricate priapulid larvae lack a functional anus; however, they nevertheless possess an anal pore (Wennberg *et al.* 2009). Steiner *et al.* (2014) argued that further characteristics warrant consideration of a cycloneuralian affinity, *viz.* the complex aperture of the theca and the invaginated derivation of the integument that comprises the aperture and theca more generally; the symmetry of the hatchlings and the presence of a large blastocoel. Indeed, cycloneuralians are united, primitively at least, in possessing a complex introvert and, hence, they are sometimes referred to as the Introverta (Nielsen 2001). Their introvert and pharyngeal scalids, and indeed their pharynx, exhibit various forms of symmetry, including the full range of three- to ninefold symmetry (Bresciani 1991; Kristensen 1991; Kristensen and Higgins 1991; Storch 1991; Wright 1991), easily encompassing the four- and fivefold symmetry exhibited by *Quadrapyrgites* and *Olivooides*, respectively. Steiner *et al.* (2014) also drew comparisons between the large open body cavity of priapulids and that of *Olivooides*, yet the body cavity of *Olivooides* embryonic stages is anything but open, divided into different regions by a complex series of walls (Dong *et al.* 2013; Han *et al.* 2013), quite unlike priapulid larvae and adults (Storch 1991). Steiner *et al.* (2014) observed that the olivooids lack an armature of introvert or pharyngeal scalids, but argued that scalids are not present in the Cambrian putative cycloneuralian larvae *Orstenoloricus shergoldii* and *Shergoldiana australiensis* (Maas *et al.* 2009). However, *Shergoldiana australiensis* does not have a lorica and the introvert is missing as a consequence of decomposition from all specimens of *Orstenoloricus shergoldii* (Maas *et al.* 2009). This is a common taphonomic feature of fossil palaeoscolecids (stem-Priapulida; Harvey *et al.* 2010), which are a common component of Cambrian–Silurian fossil assemblages (Hints *et al.* 2004), even as articulated remains (Kraft and Mergl 1989; Müller and Hinz-Schallreuter 1993; Conway Morris 1997), but which preserve the introvert in only rare circumstances of preservation (Hou and Bergström 1994; Liu *et al.* 2014*b*). The possible taphonomic absence of an armed introvert can be excluded as a possibility for the olivooids, since the anatomy of their aperture is known in fine detail and introvert scalids are demonstrably absent.

The absence of introvert scalids from the olivooids need not be, in and of itself, fatal to the hypothesis that these organisms are cycloneuralians. The aperture of *Shergoldiana australiensis* is well preserved and similarly demonstrates that neither an introvert armature nor an introvert was present in life (Maas *et al.* 2007, 2009). Indeed the aperture of *Shergoldiana* strongly resembles that of the olivooids, however, there is little evidence to substantiate its classification as a cycloneuralian. Though all introvertans (*sensu* Nielsen 2001) obviously possess an introvert, attempts to reconstruct the characteristics of the ancestral introvertan have concluded that it lacked scalids entirely, either as part of introvert of the pharynx (Schmidt-Rhaesa 1998) contra Liu *et al.* (2014*b*) and Smith & Caron (2015). This is because of fundamental differences in the anatomy of the scalids, which are comprised solely of cuticle in nematoids (Nematoda + Nematomorpha) and house nerves and vasculature in scalidophorans (Kinorhyncha + Loricifera + Priapulida). Nevertheless, an introvert and a through gut remain plesiomorphies of the Introverta and, indeed, Cycloneuralia, neither of which characters is present in the olivooids. Steiner *et al.* (2014) tried to rationalize the absence of a gut by interpreting the thecate stages as lecithotrophic larvae but, as Liu *et al.* (2014*a*) argued, this is untenable for an organism that undergoes such prolonged episodic and volumetric growth. Attempts to interpret the aperture of the olivooids as an introvert would be futile, not least since the growth zone is quite distinct from cycloneuralians: there is opening only for the gradual emergence

of integument from the theca, not for the recursive eversion and inversion characteristic of introverts. There is certainly no evidence to support the speculative hypothesis of episodic ecdysis in olivooids, as suggested by Steiner *et al.* (2014), which would imply an ecdysozoan and, therefore, a cycloneuralian affinity. Rather, all of the available evidence indicates that the integument of the embryo is retained in the theca (indeed it is a central plan of the hypothesis of association of the fossils representing embryonic and thecate stages of development), which grows, as does the embryo, through the gradual release of integument from the aperture.

In summary, the hypothesis of a cycloneuralian affinity for olivooids rests ultimately, as did the hypothesis of an echinoderm affinity, on little more than the superficial similarity of four- and five-fold symmetry, which have evolved many times in animal phylogeny. Thus, we reject the hypothesis of a cycloneuralian affinity for the olivooids.

Cnidarian affinity

With the recognition that 'Punctatus' is a component of the life cycle of *Olivooides* a cnidarian hypothesis of affinity was inevitable. This is because the thecate stage had long been considered cnidarian, through comparison to conulariids, and to hexangulaconulariids that occur in the same deposits (Conway Morris & Chen 1992; van Iten *et al.* 2010; Steiner *et al.* 2014), and which have themselves been interpreted as cnidarians. *Olivooides* shares with both groups the presence of an annulated conical test, fine longitudinal sculpture and a bluntly tapering apex with radial folds. Indeed, the comparison of *Olivooides* and hexanguloconulariids is particularly compelling since both appear to develop directly from an embryo and retain the embryonic integument (van Iten *et al.* 2010). However, the connection to cnidarians is achieved through the better-characterized conulariids that, in addition to the characters shared with olivooids and hexangulaconulariids (listed above), share with coronate scyphozoan cnidarians evidence of strobilation and the presence of a bi-layered integument comprising a conical sheath, ornament. Many conulariids and coronates also possess seriated internal tooth-like structures that project (tetra)radially into the theca from the perradii and interradii (or their topological homologues in conulariids; van Iten 1992*b*). However, many of these characters may be symplesiomorphies of medusozoan cnidarians, rather than evidence of membership of Coronata, or even Scyphozoa, in particular (Werner 1966, 1970, 1973).

The similarities shared by olivooids and conulariids provide meagre evidence on which to establish an hypothesis of affinity. However, with the discovery of an common pattern of development in the hexangulaconulariids, the linkage to conulariids and, by implication, cnidarians, is considerably better substantiated, rendering the known life cycle of olivooiids far less exceptional than it would otherwise appear in comparison to living cnidarians alone (Dong *et al.* 2013). Regardless, the data on the internal anatomy of *Olivooides* (Dong *et al.* 2013; Han *et al.* 2013) would appear to substantiate cnidarian affinity quite independently of the hexangulaconulariids and conulariids.

A stem-eumetazoan interpretation of the olivooids was based principally on an absence of evidence of a more derived affinity (Yasui *et al.* 2013), rather than compelling evidence for the absence of derived characters. Subsequent research has demonstrated the presence of characteristics of derived cnidarians (Dong *et al.* 2013; Han *et al.* 2013, in press). Within Cnidaria, olivooids have been attributed to Cubozoa (Han *et al.* 2013, in press) and Hydrozoa (Zheng *et al.* 2012*b*), in addition to Scyphozoa (Bengtson & Yue 1997; Yue & Bengtson 1999*a*; Dong *et al.* 2013). The hypothesis of a cubozoan affinity is based foremost on the internal anatomy of embryos of *Olivooides* and *Quadrapyrgites,* which is an over-interpretation of the fossilized features made using the cubozoan medusa as an interpretative model (Han *et al.* 2013, in press). This approach, which is not justified *a priori*, inevitably gives the conclusion that *Olivooides* is allied with cubozoans (cf. Donoghue & Purnell 2009). However, a sexually mature medusa is clearly an inappropriate interpretative model for what is evidently a benthic thecate organism that, if a cnidarian affinity is at all tenable, must represent a polyp. However, as Liu *et al.* (2014*a*) observed, the polyp stage is very much reduced in extant Cubozoa, lacking the mesenteries that are evidently present from the internal anatomy of *Olivooides* embryonic stages (Han *et al.* 2013); evidence of mesenteries in Cambrian putative cubozoans (cf. Han *et al.* 2010) is moot since their assignment to Cubozoa is equally dubious. The assignment of olivooids to Scyphozoa and Hydrozoa is better substantiated, based on the common presence of a theca in the polyp stage in Hydrozoa (Zheng *et al.* 2012*b*), and the somewhat greater similarity in the nature of the ornament of the theca in coronate Scyphozoa (Bengtson & Yue 1997; Yue & Bengtson 1999*a*; Steiner *et al.* 2006; Dong *et al.* 2013; Liu *et al.* 2014*a*); structures resembling strobilating ephyrae provide further support for this comparison (Dong *et al.* 2013). Had the link between the theca of *Olivooides* (as 'Punctatus'), hexangulaconulariids and conulariids not been established prior to the life cycle of *Olivooides*, it is doubtful that the olivooids would have been allied so precisely with Coronata. The preserved internal anatomy of *Olivooides* embryos does not serve us such precision since its interpretation is equivocal at

present, at least beyond the identification of meduzoan or cnidarian symplesiomorphies or synapomorphies.

Steiner *et al.* (2014) rejected the cnidarian hypothesis of affinity for olivooids on the basis that many of their characters are atypical of cnidarians, for instance the presence of a stellate embryonic integument, yet systematics has long rejected autapomorphies as indicative of phylogenetic affinity (Hennig 1950). Steiner and colleagues were concerned also for the lack of a planula larva and of proximal attachment structures in the theca, yet since the thecate stage develops directly from an embryo, rather than a planula larva, as it evidently also does in hexangulaconulariids (van Iten *et al.* 2010), these absences are to be expected. They claim that the pentaradial symmetry of *Olivooides* is not compatible with cnidarians, which are usually (but not exclusively) tetra- or hexaradial. Yet as we have already observed, cnidarians exhibit a great many patterns of symmetry, and the commonalities shared by pentaradial *Olivooides* and tetraradial *Quadrapyrgites* remove all such concerns over the organizational symmetry of olivooids considered to be cnidarians (Dong *et al.* 2013). Steiner *et al.* (2014) also highlighted the absence of internal septae or denticle circlets in the thecae of scyphozoans and conulariids from olivooids, yet these structures are not present in all scyphozoans or conulariids (Dong *et al.* 2013). Steiner *et al.* (2014) were further concerned by the manner in which an alimentary canal develops from an archenteron, itself developing from an invagination of ectoderm, through the early stages of aperture development. However, these concerns are unfounded since they are based on the incorrect interpretation of these embryonic stages as representing gastrulae when, as has been shown from the preserved internal anatomy (Dong *et al.* 2013; Han *et al.* 2013, in press), organogenesis has evidently been underway since long before these embryonic stages.

Concerns over the interpretation of the olivooids as cnidarians can be laid to rest, and instead we turn our attention to resolving the affinity of the olivooids among total-group Cnidaria, on the basis that it is the only prior hypothesis of affinity that has withstood scrutiny. In so doing we follow Han *et al.* (in press) in considering the affinity of the olivooids within the cladistic dataset compiled by Marques & Collins (2004) and augmented by van Iten *et al.* (2006). However, we do not follow the codings for the olivooids presented in Han *et al.* (in press) in an attempt to avoid the circularity of interpreting their anatomy on a cubozoan model and then attempting to discriminate an affinity among Cnidaria as though the interpretations of olivooid anatomy will not lead inevitably to a cubozoan affinity. Indeed, it is our view that the majority of anatomical homologies identified by Han *et al.* (2013, in press) could not be discriminated as fossilized biological structures except through the lens of an adult cubozoan medusa interpretative model. Rather, in interpreting the anatomy of *Olivooides*, and of the olivooids more generally, we assume only membership of total group Cnidaria and then draw comparisons between characterized structures and their potential homologies among component cnidarian clades and grades. In this, we interpret the embryo as a developing thecate polyp since the preserved structures are incompatible with a medusa. The stellate and striate tissues are interpreted as the external periderm, and the inner walls as additional structural, presumably ectodermal tissue layers, all of which were unmineralized and flexible in life. We consider the pentagonal axial structure as support for the mesenteries, the most abapertural radial wall as, perhaps, enclosing the mesenteries adaperturally, and we draw comparisons between the paired pentaradial projections and the 'teeth' in coronate scyphozoan polyps (Werner 1983; Jarms 1991), where they appear to serve a role in anchoring the polyp within the theca. We coded *Olivooides* and *Quadrapyrgites* for the characters described in van Iten *et al.* (2006) based particularly on the evidence presented here for *Olivooides* and for the embryonic stages of *Quadrapyrgites* presented in Steiner *et al.* (2014) and Han *et al.* (in press), adding only one character relating the presence of peridermal teeth in the thecae of coronate scyphozoans (Jarms 1991) and the olivooids, absent from all other taxa considered.

Phylogenetic analysis of the dataset using the branch and bound search algorithm within PAUP* 4.0a146 (Swofford 2002) yielded just 10 MPTs at 127 steps (CI, 0.74; RI, 0.74; RCI, 0.55) that differed only in terms of the relationships among Hydrozoa (Fig. 10A). Coronates comprise an outgroup to a clade of olivooids, with conulariids and Rhizostomeae + Semaeostomeae comprising successive sister lineages within Scyphozoa. Reanalysis of this same dataset using the Goloboff criterion ($K = 2$) recovered just four MPTs at 127 steps (CI, 0.74; RI, 0.74; RCI, 0.55) that differ only slightly in terms of the relationships among Hydrozoa (Fig. 10B).

These results indicate that the olivooids comprise a clade of total group Coronata within crown-Scyphozoa. As such, the olivooids evidence direct development and variation in the radial symmetry of Scyphozoa, not commonly encountered in extant members of the clade. It would be tempting to conclude, given their antiquity, that these fossils reflect the plesiomorphy of direct development among Scyphozoa, that indirect development from embryo to polypoid adult is a derived, perhaps convergent phenomenon among extant medusozoan cnidarians (Bengtson & Yue 1997; Conway Morris 1998; Yue & Bengtson 1999*a*). However, this meager evidence is diminished by our demonstration of a close phylogenetic relationship between *Olivooides* and *Quadrapyrgites*, leading to inference that direct development has evolved in

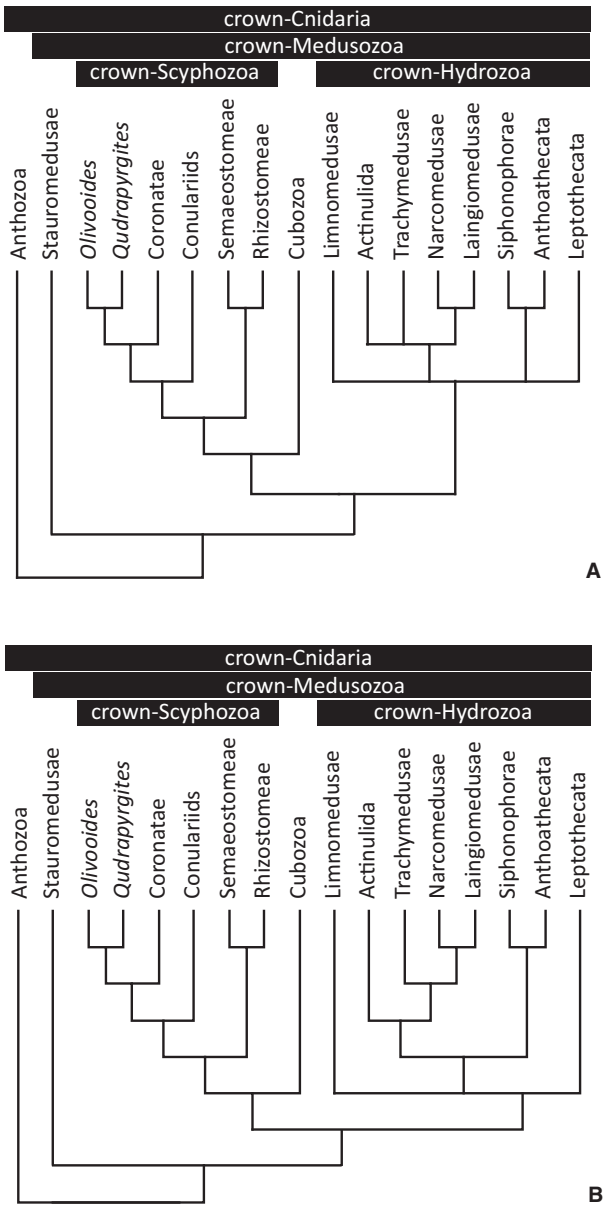

FIG. 10. The phylogenetic affinity of the olivooids *Olivooides* and *Quadrapyrgites*. A, strict consensus of 10 MPTs derived from parsimony analysis of the cladistic data set. B, strict consensus of four MPTs derived from parsimony analysis of the same data set but implementing the Goloboff search criterion with a concavity constant value $K = 2$. In both instances, the olivooids are resolved as total-group Coronata, crown-Scyphozoa.

the lineage leading to the olivooids after their separation from that leading to crown-Scyphozoa. Nevertheless, our results support the view that the patterns of cnidarian symmetry and life history strategy represented in the modern biota are but a subset of those that have existed. This need not reflect profound changes, such as the canalization of embryological programs but, merely, the winnowing away of variation by stochastic extinction across the diversity and disparity of Cnidaria through their evolutionary history.

CONCLUSIONS

We reviewed interpretations of developmental stages of the Cambrian olivooids *Quadrapyrgites* and, particularly, *Olivooides*. We showed that embryology is characterized by the development of a theca, which is commonly preserved as a consequence of its peridermal sheath. Variation in the morphology of embryonic stages reflects the release of peridermal tissue at the aperture, formed within the theca. Internally, the embryo is comprised of a series of additional skeletal tissue layers that form incomplete radial walls that divide the space within the theca. These include the abapertural region which housed the mesenteries of a polyp and peridermal teeth. The aperture of the theca opened episodically to release new peridermal tissue, *in ovo* and in post-embryonic development. Thus, the aperture was capable of opening and closure, but presumably it remained open for protracted intervals to allow the polyp to feed. We rejected the interpretation of the olivooids as cycloneuralians on the grounds that they lack an anus and an introvert, in embryo and adult. Indeed, the available evidence indicates that the olivooids were eumetazoan diploblasts. Many of the characters that have been marshalled to support a cubozoan affinity for the olivooids are based on interpretation of structures that are likely to be diagenetic artefacts. Phylogenetic analysis supports the interpretation of the olivooids as comprising a clade of total group Coronata within crown-Scyphozoa. Hence, they evidence greater variation (if not plesiomorphy) of life history strategies and body symmetry in Cambrian cnidarians than is manifested in extant members of the phylum.

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DATA ARCHIVING STATEMENT

Tomographic data for this study are available in the Bristol Digital Repository (Dong *et al.* 2016*a*): http://dx.doi.org/10.5523/bris.ig22c0fcvoen1qrvpuoctgij9

Phylogenetic data for this study are available in the Dryad Digital Repository (Dong *et al.* 2006b): http://dx.doi.org/10.5061/dryad.bp685

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